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Light Reduces the Excitation Efficiency in the *nss* Mutant of the Sheep Blowfly *Lucilia*

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ABSTRACT The *nss* (no steady state) phototransduction mutant of the sheep blowfly *Lucilia* was studied electrophysiologically using intracellular recordings. The effects of the *nss* mutation on the receptor potential are manifested in the following features of the light response. (*a*) The responses to a flash or to dim lights are close to normal, but the receptor potential decays close to the baseline level during prolonged illumination after a critical level of light intensity is reached. (*b*) The decline of the response is accompanied by a large reduction in responsiveness to light that recovers within 20 s in the dark. (*c*) The full reduction in responsiveness to light is reached when ~13% of the photopigment molecules are converted from rhodopsin (R) to metarhodopsin (M). (*d*) A maximal net pigment conversion from R to M by blue light induces persistent inactivation in the dark, without an apparent voltage response. This inactivation could be abolished at any time by M-to-R conversion with orange light. The above features of the mutant indicate that the effect of the *nss* mutation on the light response of *Lucilia* is very similar to the effects of the transient receptor potential (*trp*) mutation on the photoreceptor potential of *Drosophila*. Noise analysis and voltage measurements indicate that the decay of the receptor potential is due to a severe reduction in the rate of occurrence of the elementary voltage responses (bumps). The bumps are only slightly modified in shape and amplitude during the decline of the response to light of medium intensity. There is also a large increase in response latency during intense background illumination. These results are consistent with the hypothesis that separate, independent mechanisms determine bump triggering and bump shape and amplitude. The *nss* mutation affects the triggering mechanism of the bump.

INTRODUCTION

Our knowledge of invertebrate phototransduction has advanced considerably in the last few years (Brown et al., 1984; Fein et al., 1984; Johnson et al., 1986; Devary et

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al., 1987; for reviews, see Stieve, 1986; Paulsen and Bentrop, 1986; Payne, 1986; Tsuda, 1987). Nevertheless, there are still wide gaps and some confusion in our understanding of this complex multistage process.

One of the promising approaches for studying phototransduction in invertebrates is based on the existence of several phototransduction-defective mutants of *Drosophila* (Pak, 1979). One of the intensively studied mutants is the *trp* (transient receptor potential) mutant (Cosens and Manning, 1969; Minke et al., 1975; Minke, 1979, Minke, 1982; Lo and Pak, 1981; Montell et al., 1985). The *trp* mutant is characterized by the following features. (*a*) The receptor potential, in response to a short flash or to dim light, seems to be close to normal; however, during prolonged intense illumination, the response quickly decays to baseline. The ability to respond to light again recovers in the dark within 1 min after the light is turned off (Minke, 1982). (*b*) The structure of the eye and the photopigment properties of the mutant seem to be normal at eclosion, but begin to show some deterioration after ~5 d (at 24°C) (Cosens and Perry, 1972; Minke, 1982). (*c*) The phenotypic expression of the mutant depends on the developmental temperature: the phenotype of the mutant is more similar to that of the normal fly when the fly is raised at 19°C as compared with 25°C (Minke, 1983). (*d*) Electrophysiological measurements of the intensity response function and of the response latency at various background illuminations reveal that the decline of the receptor potential during prolonged illumination is not accompanied by the typical features of light adaptation (Minke, 1982). The size of the quantum bumps also does not seem to be much reduced during the decline of the receptor potential (Minke et al., 1975). A large reduction in the extracellular Ca^{2+} level, which presumably reduces the intracellular Ca^{2+} level, makes little difference to the mutation phenotype (Minke, 1982). Taking together the above observations, it was suggested (Minke, 1982) that the decay of the *trp* response during intense illumination arises neither from a reduction in the available photopigment molecules nor from light adaptation, but rather from a light-induced reduction in excitation efficiency owing to some defect in an unknown intermediate stage of phototransduction.

The *trp* gene of *Drosophila* was recently isolated and its identity was confirmed by complementing the mutant trp^{CM} allele of the *trp* gene by P-element–mediated germline transformation of a 7.1-kb DNA fragment that rescued the *trp* mutant; however, the role of the *trp* gene product is still unknown (Montell et al., 1985). To study the function of the *trp* gene product, we have initiated detailed pharmacological and biochemical studies of the *trp* mutant. From these studies, it has become apparent that intracellular recordings and noise measurements from single photoreceptors of the *trp* mutant during application of test compounds is technically not feasible because of the tiny diameter (~3 μm) of *Drosophila* photoreceptors (Suss, E., S. Barash, D. G. Stavenga, H. Stieve, A. Selinger, and B. Minke, manuscript in preparation); also, measurements of light-induced binding of $[S^{35}]GTP_{\gamma}S$ and GTPase activity in crude membrane preparations of *Drosophila* eyes showed only small differences between light and dark measurements relative to similar measurements in the larger flies *Musca* and *Lucilia* (Heichal, O., M. Lowe, A. Blumenfeld, B. Minke, and Z. Selinger, manuscript in preparation). In order to verify the results obtained by recordings of the electroretinogram (ERG) and by measurements in purified mem-

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brane preparations of the *trp* mutant, we have studied a mutant of the sheep blowfly, *Lucilia cuprina,* that shows important similarities to the *trp* mutant. This mutant, called *nss* (no steady state), which was discovered by Howard (1982), is technically more accessible because of the larger diameter of its photoreceptors. We could perform intracellular recordings and noise measurements in this mutant for long periods of time and during the application of test compounds. We also succeeded in measuring light-induced biochemical reactions in crude membrane preparations of fly eyes in which the physiological properties of light-activated enzymes were presumably minimally modified.

In the present report, we describe the characteristics of the receptor potential of the *nss* mutant. In particular, we provide the basic data leading to the conclusion that the *nss* mutant has essentially the same phenotype as the *trp* mutant. The detailed knowledge of the effects of the *nss* mutation on the light response of *Lucilia* photoreceptors confirms and complements our studies of the *trp* mutant. We also examine whether the decline of the *nss* response to lights of long duration arises primarily from a reduction in the rate of occurrence of the quantum bumps or from changes in the shape and amplitude of bumps. Establishing a similarity between the *trp* and *nss* mutants should be of great interest to investigators who are looking for an accessible invertebrate preparation in which to study molecular mechanisms of bump initiation.

MATERIALS AND METHODS

White-eyed *L. cuprina* and its white-eyed mutant *nss* were obtained from Dr. G. G. Foster, CSIRO, Division of Entomology, Canberra, Australia. Flies were immobilized by cooling for 2 min and then mounted with wax on a rotating stage, dorsal side up. The upper part of the cornea was sliced off with a vibrating razor blade to expose a small hole in the dorsal part of the eye, which was covered with petroleum jelly.

Recordings and Power Spectra Calculations

Intracellular recordings were made by inserting into the retina 2 M KCl–filled micropipettes with resistance of 100–150 MΩ. The indifferent electrode was a broken pipette (5-μm tip diameter) filled with fly Ringer's solution containing (millimolar): 140 NaCl, 2 KCl, 2 $CaCl_2$, and 5 $MgCl_2$. The pH was kept at 7.0 using a 10 mM HEPES buffer. The indifferent electrode was placed close to the recording electrode to minimize interference from the ERG. The electrical responses were amplified by 100 and low-pass-filtered with a differential amplifier (26A2, Tektronix, Inc., Beaverton, OR) with a 3-dB point at 1 kHz. The amplified and filtered responses were sampled with an LSI 11/23 microcomputer (Digital Equipment Corp., Maynard, MA) and stored on floppy disks. The rate of sampling was 500 per second; this is sufficient for the bandwidth of the signal, which is below 300 Hz (Minke and Stephenson, 1985). In some experiments, in order to further filter the background noise, the following procedure was used. The sampling rate was set at 2,000 (or 4,000) samples/s, the samples were grouped into sets of four (or eight) consecutive samples each, and the averages of these sets were retained instead of the original samples. (This procedure is equivalent to low-pass-filtering.) Power spectra were calculated by fast Fourier transform from blocks of 1,024 points. The power spectra of several (usually 10) such consecutive, nonoverlapping blocks

were averaged. The averaged spectra were further smoothed by a moving n-points average, with $n \leq 31$.

Light Stimulation

The light source consisted of a 100-W, 12-V halogen lamp in conjunction with two KG-3 heat filters, an OG-590 edge filter, or a BG28 blue filter (all from Schott Glass Technologies, Inc., Mainz, Federal Republic of Germany). The light intensity was attenuated by neutral density filters (Ditric, Marlboro, MA). The unattenuated blue and orange light intensities at the level of the eye were 2.6 and 14 mW/cm^2, respectively. We also used a 150-J photographic flash (Broncolor, Bron Elektronic, Allschwil Switzerland) in conjunction with an OG-570 edge filter (Schott Glass Technologies, Inc.) The intensity of the flash was such that three orange flashes were sufficient to convert almost all the photopigment molecules existing in the metarhodopsin (M) state back into the rhodopsin state as measured by the M potential (see Fig. 8). The timing of the recordings and stimulation was controlled by a microprocessor-controlled eight-channel pulse-generator (Master-8, AMP Instruments, Jerusalem, Israel).

RESULTS

A Comparison Between the Receptor Potentials of Normal Lucilia and the nss Mutant

The receptor potentials of the normal *Lucilia* in response to increasing intensities of orange lights are shown in Fig. 1. Orange lights were used in these experiments to prevent the induction of persistent excitation, which is induced by net pigment conversion from rhodopsin to metarhodopsin by intense blue light. This excitation, which is called the prolonged depolarizing afterpotential (PDA), continues in the dark long after the intense blue light is turned off and makes the interpretation of the light response more complicated. The receptor potential of Fig. 1 is a typical arthropod-type response. At very dim lights, the response is composed of unitary depolarizations (quantum bumps), which fuse and form a noisy depolarization when the light intensity is increased (Dodge et al., 1968). With a further increase in light intensity, an initial transient appears that declines to a lower "steady state" phase. The steady state phase of the receptor potential slowly declines during an additional 10–30 s and then remains constant as long as the light stays on. The steady state phase of the response to a stronger light is more depolarized, but its noise level is reduced because of light adaptation (Dodge et al., 1968).

The receptor potential of the *nss* mutant to steps of dim lights is very similar to that of normal *Lucilia,* except that the noise level is often a little larger (Fig. 2 *A*, lower traces). Occasionally, discrete waves with relatively large amplitudes are observed (arrows). A detailed analysis of these discrete waves is outside the scope of this work. In general, these large events might be regenerative depolarizations since they have a rather uniform amplitude and show many similarities to the regenerative bumps appearing in vivo in *Limulus* lateral eye (Barlow and Kaplan, 1977). When the light intensity is increased, a very narrow range of intensities is reached, in which the receptor potential declines to a constant steady state level of a few millivolts amplitude, which is lower than that reached with weaker lights (compare the responses to the relative intensities 2.5 and 2.3). The small steady state depolarization still shows considerable voltage fluctuations (noise) as long as the light is on

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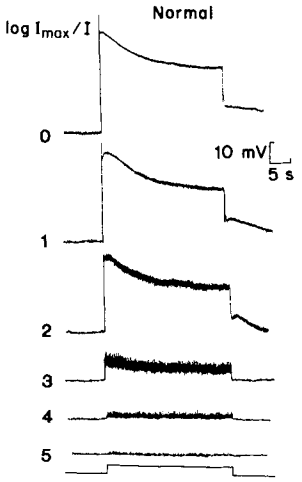

FIGURE 1. Receptor potentials of normal *Lucilia* in response to increasing intensities of orange (OG-590 edge filter) lights as indicated. All responses are intracellular recordings from a single cell. The bottom trace is the light monitor.

(Fig. 2 *A*, log $I_{max}/I = 2.3$). The persistence of this noisy depolarization was confirmed for a light duration of 8 min (not shown). An additional slight (0.15 log) increase in light intensity was sufficient to reduce the steady state phase of the response significantly below the original dark baseline and to depress the noise completely after ~12 s (Fig. 2 *A*, log $I_{max}/I = 2.15$). In many cells, after this decline, the

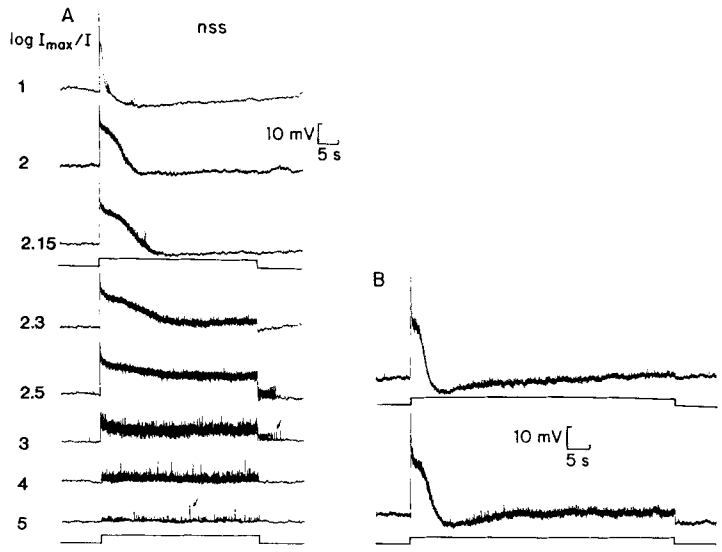

FIGURE 2. (*A*) Receptor potentials of the *nss* mutant of *Lucilia* in response to increasing intensities of orange (OG-590 edge filter) lights as indicated. Arrows indicate regenerative unitary events. All responses were recorded from a single cell. (*B*) Intracellular records from a single cell of another *nss* mutant in which the response declined temporarily below baseline and rose again to give noisy depolarization during light. The relative intensities of the two orange (OG-590 edge filter) light pulses were log $I_{max}/I = 1.65$ and 1.8 for the upper and lower traces, respectively.

continuing light did not evoke any voltage response; however, in other cells, a low level of depolarization accompanied by an elevated noise level slowly appeared (Fig. 2 *B*). Steps of yet higher light intensity (Fig. 2 *A*) caused the response to decline faster, usually followed by a larger transient hyperpolarization phase, and the noise, in all cells, was similar to the noise in the dark.

The Decay of the nss Response Involves a Reduction in the Rate of Occurrence of the Bumps

Two mechanisms can account for a decline of the receptor potential during prolonged illumination: a reduction in bump size or a reduction in their rate of occur-

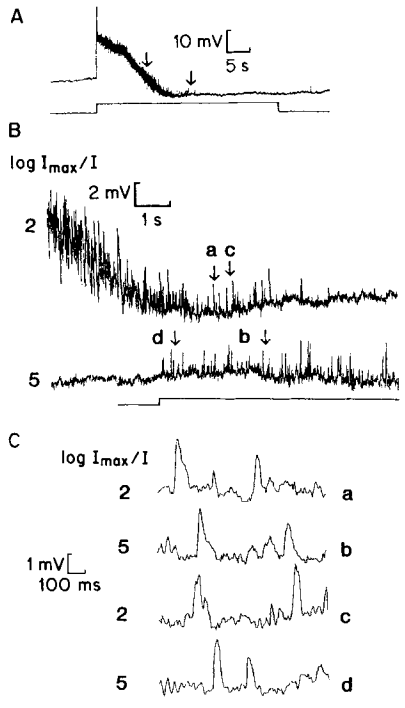

FIGURE 3. The decline of the receptor potential of the *nss* mutant is accompanied by a reduction in the rate of occurrence of the bumps, with little or no change in their amplitude and shape. (*A*) A receptor potential of the *nss* mutant in response to orange (OG-590) light with maximal intensity attenuated by 2 log units (log $I_{max}/I = 2.0$). The response was recorded from the same cell as in Fig. 2 *A* at the beginning of the experiment before the other responses were recorded. (*B*) Upper trace: a magnified time region of the response shown in *A* (between two arrows). Lower trace: a magnified time region of a response recorded from the same cell but in response to dim orange light with maximal intensity attenuated by 5 log units (log $I_{max}/I = 5.0$). (*C*) Magnified time regions of the two responses shown in *B* at the times indicated in *B* by the arrows. The letters *a–d* (right) correspond to the letters in *B* near the arrows.

rence (Rushton, 1961; Dodge et al., 1968; Wong and Knight, 1980; Wong et al., 1982). It was suggested, but not rigorously shown that the decay of the response in the *trp* mutant of *Drosophila* is due to a reduction in the rate of occurrence of the bump (Minke et al., 1975).

One of the major advantages of the *nss* mutant of *Lucilia* over the *trp* mutant of *Drosophila* is the possibility of recording membrane potentials for long periods of time and with a good signal-to-noise ratio. Accordingly, single bumps are observed during dim lights and also after the decay of the receptor potential to near baseline. Fig. 3 *B* (lower trace) presents the receptor potential of the *nss* mutant in response

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to dim light ($\log I_{max}/I = 5.0$) and the response of the same cell observed during 1,000 times more intense light ($\log I_{max}/I = 2.0$). The amplitude and shape of the quantum bumps that compose the response to the dim light are clearly observed, at high gain, in a magnified fraction of the responses (Fig. 3, *B* and *C*). The decaying response to the intense light (Fig. 3 *A*) is a noisy transient depolarization, which resolves into discrete depolarizations when the response reaches baseline (Fig. 3 *B*, upper trace). The bumps observed at this phase are very similar (qualitatively) in amplitude and shape to those observed during dim light (Fig. 3 *C*).

Bumps of similar shapes and amplitudes are recorded during very dim light and during the decline of the response to a much stronger light. This suggests that the rate of occurrence of the quantum bumps diminishes monotonically, during intense light, until it is so low that individual bumps can be observed. This interpretation is strengthened by the analysis of the light-induced noise of the *nss* (see below). In normal *Lucilia,* the situation is, of course, completely different. As shown in Fig. 1, individual bumps are observed in the response to dim light ($\log I_{max}/I = 5.0$). However, an increase of the stimulus intensity gives a large depolarization with reduced noise in which individual bumps can no longer be discriminated from the noise.

The Characteristics of the Light-induced Noise in the nss Mutant

The power spectrum of the light-induced noise characterizes the frequency decomposition of this noise and, in steady state conditions, reflects the rate as well as the amplitude and shape of the elementary voltage events that compose the noise. Accordingly, the power spectrum is a powerful tool for the comparison between the light-induced noise of the mutant and the normal fly. It also enables (assuming linearity and stationarity) a comparison between the elementary voltage events underlying the mutant response to dim light and to light that causes the decline of the response to near the baseline.

Fig. 4 presents power spectra that were calculated from the noise of the receptor potential at the steady state phase 20 s after light onset in normal *Lucilia,* during 80 s illumination with increasing intensities of orange lights. The power spectra of normal *Lucilia* depend in a characteristic way on light intensity: (*a*) The variance spectral density increases at all frequencies at dim lights (up to $\log I_{max}/I = 4.0$; Fig. 4, left). (*b*) The variance spectral density decreases at low frequencies and increases at high frequencies at intense lights (Fig. 4, right). These characteristics are illustrated by the systematic reduction in the power spectra curves in the low frequency range when the light intensity was increased (above $\log I_{max}/I = 4.0$) and by the observation that the curves, calculated from responses to intense lights, cross the curves calculated from responses to dimmer lights at high frequencies. This "curve crossing" was found in all the normal flies studied. Calculations of power spectra from the same preparation by Howard et al. (1987) gave similar results. A detailed account of the quantitative changes in the power spectrum will appear in a later publication (Barash S., E. Suss, and B. Minke, manuscript in preparation). In general, the above changes in the power spectrum reflect the reduction in bump amplitude and duration at high light intensities owing to light adaptation (Wong and Knight, 1980; Wong et al., 1982).

The power spectra calculated from the light-induced noise of the *nss* responses are presented in Fig. 5 *A*. The power spectra calculated from the noise superimposed on responses to very dim lights are similar to those of the normal *Lucilia* (Fig. 5 *A*, left). However, during lights that caused a decline of the light response toward baseline, the power spectrum became similar to that calculated for a very dim light (Fig. 5 *A*, right). For example, the power spectrum calculated from the response to light of relative intensity log I_{max}/I = 2.0 resembles the power spectrum calculated from the response to very dim light (log I_{max}/I = 5.0) more closely than it does the power spectrum of the response to light of medium intensity (log I_{max}/I = 3.0), which did not cause any decline toward baseline. The power spectrum calculated from the response to relative light intensity of log I_{max}/I = 1.0 (after the decline to baseline level) was similar to the power spectrum calculated from the noise in the dark (not shown). The differences among the curves at low (<3 Hz) frequencies are

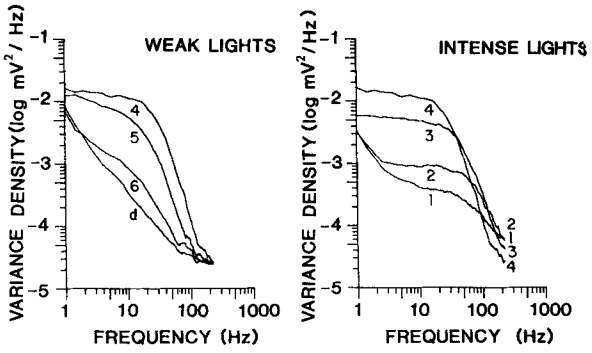

FIGURE 4. Power spectra calculated from the steady state phase of the receptor potential of normal *Lucilia* 20 s after light onset, from segments of 30 s duration in response to increasing intensities of orange (OG-590) lights (see Methods). (*Left*) The bottom curve was calculated from the noise in the dark ("d"). The other curves (above) were calculated from responses to increasing intensities of orange lights in the range between relative intensities log I_{max}/I = 6.0 and 4.0 in steps of 1 log unit. (*Right*) Power spectra calculated from the noise of the same cell in response to more intense lights in the range between relative intensities log I_{max}/I = 4.0 (upper curve) and 1.0, in steps of 1 log unit. All power spectra were calculated from responses of a single cell.

not significant. They were influenced by vibrations of the tissues of the live flies from which the recordings were made.

Derivation of the Effective Bump Duration from the Power Spectra

To facilitate a quantitative comparison between the shape and duration of the quantum bumps induced by dim and intense lights, we used the theory and procedure of Wong and Knight (1980) in our experiments (see also Wong, 1978, and Wong et al., 1982). In detailed and comprehensive studies of the *Limulus* lateral and ventral eyes, Wong and Knight (1980) and Wong et al. (1982) estimated the bump shape and duration from the power spectrum of the steady state voltage (or current) responses to light in the following way. They assumed that the noisy receptor potential is composed of superimposed bumps having a common waveform, $B(t)$. They furthermore assumed an analytic form for the bump shape known as the Γ

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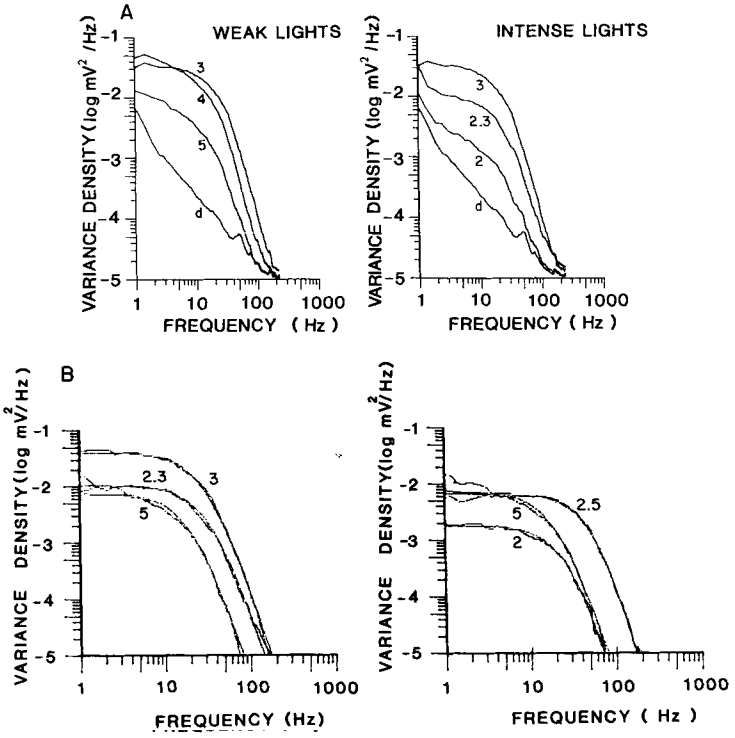

FIGURE 5. (*A*) Power spectra calculated from the receptor potential of the *nss* mutant in response to increasing intensities of orange (OG-590) lights as in Fig. 4. The power spectra were calculated from 10 consecutive nonoverlapping 2,048-s segments, beginning 15 s after the light was turned on. All power spectra were calculated from responses of a single cell. (*Left*) The bottom curve was calculated from the dark noise ("d"). The other curves were calculated from responses to increasing intensities as indicated on the relative log scale. (*Right*) Power spectra calculated from the same cell in response to lights that elicited decaying responses. The upper curve is the same curve presented on the left for comparison. The variance density at low frequencies (<3 Hz) showed variations in different experiments. The peak at 50 Hz ("d") is an artifact, being the first harmonic of the power line frequency. The power spectrum of relative light intensity 2 was calculated from the response in Fig. 3 *A*. (*B*) The power spectra can be very well fitted by Eq. 2. The power spectrum calculated from the noise in the dark was subtracted from the power spectra calculated from the noise in the responses to lights of various intensities of the cell of Fig. 5 *A*. The relative intensities of the light stimuli are indicated in log units. In each pair of curves, the smooth curve is a plot of Eq. 2 that best fitted the experimental curve and the noisier curve is the calculated power spectrum. Power spectrum 5 crosses both power spectra 2.3 and 2.5 at low frequencies. This crossing is not significant since it showed variations in different experiments. The parameters A, τ, and $n + 1$, which were used to plot Eq. 2, are listed in Table II. When A, τ, or $n + 1$ were modified by more than ±5%, the fitted curve clearly deviated from the experimental one.

distribution:

$$\Gamma(t; n, \tau) \equiv \frac{1}{n!\tau}(t/\tau)^n e^{-t/\tau}. \quad (1)$$

Eq. 1 yielded, upon Fourier transformation, the following:

$$|\tilde{B}(f)|^2 = |\tilde{\Gamma}(f; n, \tau)|^2 = \frac{1}{[1 + (2\pi\tau f)^2]^{n+1}}, \quad (2)$$

where f is the frequency; n and τ can be evaluated by fitting Eq. 2 to the scaled experimental power spectrum. The bump duration T is defined by the elementary integrals of Eq. 3:

$$T = \frac{\left[\int_0^\infty dt\, B(t)\right]^2}{\int_0^\infty dt\, B^2(t)}, \quad (3)$$

which yields

$$T = \tau \cdot \frac{(n!)^2\, 2^{2n+1}}{(2n)!}. \quad (4)$$

For details, see Wong and Knight (1980) and Schnakenberg and Wong (1986).

TABLE I

The Effective Bump Duration, T, of Normal Lucilia at Various Light Intensities

$\log I_{max}/I$	A	τ	$n + 1$	T
	log mV^2/Hz	*ms*		*ms*
6	−3.15	8.10	1.60	26.97
5	−2.10	7.85	1.75	28.22
4	−1.87	3.95	2.55	18.87
3	−2.2	2.08	3.00	11.09
2	−3.09	1.10	3.05	5.92
1	−3.55	1.10	2.60	5.33

T was calculated using Eq. 4. The parameters A (a scaling factor), τ, and $n + 1$ were determined by fitting Eq. 2 to the power spectra of Fig. 4 after the power spectrum of the dark noise ("d") was subtracted from the various power spectra.

The application of Eqs. 1–4 to calculate the effective bump duration, T, from the *nss* responses requires that steady state responses be used. Fig. 2 *A* shows that this requirement is fulfilled in responses to lights of dim and medium intensity (up to $\log I_{max}/I = 2.3$). To verify the steady state conditions, we calculated the power spectra from the responses in the dim and medium intensity range twice for each light intensity, after 10 and 15 s (from light onset), and obtained very similar power spectra in the two cases.

We used the right-hand side of Eq. 2 to approximate the power spectra calculated from the light responses. The approximation, used here on fly data (see also John-

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son and Pak, 1986) turned out to be remarkably good (Fig. 5 *B*; Barash et al., manuscript in preparation). One difference relative to *Limulus* (Wong, 1978) was, however, that the parameter n could usually be fitted better by non-integer values. By using integer values of n, we obtained considerably worse fits. In order to calculate T in these conditions according to Eq. 4, the function n was interpolated by the Γ function (Davis, 1964). The results of this approximation are presented in Tables I and II.

Tables I and II show a systematic shortening of the effective bump duration, T, with the increase in stimulus intensity in both the normal and mutant flies over the range of dim light intensities. The effective bump duration of the *nss* was somewhat longer (in most cells) than that of the normal fly at a similar stimulus intensity. The main difference in T between the mutant and the normal flies was revealed at medium light intensities, which caused a decline of the *nss* response toward baseline (log I_{max}/I = 2.5 and log I_{max}/I = 2.3). While the T of the normal fly continued to decrease, reaching a limiting value of ~5 ms at high intensities, the effective bump duration of the mutant reached a minimum of ~14 ms at a light intensity at which

TABLE II

The Effective Bump Duration, T, of the nss Mutant at Various Light Intensities

log I_{max}/I	A	τ	$n + 1$	T
	log mV^2/Hz	*ms*		*ms*
5	−2.20	8.80	2.13	36.93
4	−1.55	6.40	2.65	31.40
3	−1.49	5.80	2.20	24.93
2.5	−2.23	3.00	2.60	14.53
2.3	−2.06	5.28	2.18	22.54
2	−2.80	5.90	2.45	27.41

T was calculated using Eq. 4. The parameters A (a scaling factor), τ, and $n + 1$ were determined by fitting Eq. 2 to the power spectra calculated from the responses of Figs. 2 *A* and 3 *A* after the power spectrum of the dark noise was subtracted (see Fig. 5 *B*).

the mutation phenotype appeared and then increased to values approaching the bump duration observed with dim lights.[1]

The shortening of the bump duration with the increase in stimulus intensity is a well-known effect of light adaptation (Dodge et al., 1968; Wong, 1978; Stieve, 1986). Table II shows that there is some effect of light adaptation on the bump of the mutant, but this effect is reduced at light intensities that cause the decline of the response to baseline. The similarity in the effective bump duration at log I_{max}/I = 5.0 and log I_{max}/I = 2.0 is consistent with the similarity of bump shapes in the

[1] Although the response to light intensity of log I_{max}/I = 2.0 (Fig. 3 *A*) had no steady state phase, still, after 15 s from light onset, the change in the bump rate was relatively slow and therefore presumably introduced only a small error in the value of T, which is presented in Table II. The excellent fit of Eq. 2 to the power spectrum in Fig. 5 *B* (2) supports this conclusion. A similar excellent fit of Eq. 2 to the power spectra of Fig. 4 (after subtraction of trace "d") was also obtained (not shown).

responses to dim and stronger lights of Fig. 3 *C*. Calculations of T as a function of light intensity in the mutant and in the normal fly were performed for five different mutants and four normal flies. The calculations gave results very similar to those displayed in Tables I and II, (in one normal fly, T, under dim light, was 36.2 ms, as in the *nss* mutant).

Although the use of Campbell's theorem for estimating bump rates and amplitudes has become routine, we did not use it to calculate the bump rate and amplitude of the *nss* mutant. There are two reasons why the use of the theorem is not appropriate to the present study. First, Campbell's theorem is most useful when the importance of approximation error can be reduced by obtaining steady state responses over a large range (typically several orders of magnitude) of stimulus intensities. In the case of the *nss*, however, only a very narrow range of light intensities is strong enough for the phenotype of the mutant to be expressed, but weak enough for a steady state phase of the response above baseline to exist. Second, the *nss* responses, in the range of intermediate intensities, are usually followed by a considerable hyperpolarizing afterpotential (for instance, see log $I_{max}/I = 2.3$, 2.15 in Fig. 2 *A*). This afterpotential indicates the presence of additional processes contributing to the receptor potential. This finding conflicts directly with the major assumption necessary for application of the theorem and introduces a large error in using the response mean needed for the calculations. These complications prevent a straightforward application of Campbell's theorem to the present data, but do not altogether rule out this general approach to obtaining quantitative data. A recent extension of the theorem accommodates the contribution of more than one process to the receptor potential (Barash et al., manuscript in preparation), and also promises to be applicable to non–steady state responses (Fesce et al., 1986).

Recovery of Responsiveness of the nss Mutant in the Dark

The receptor potential of the *nss* mutant declines to baseline within 1 s of the onset of intense light. When the light is turned off, a receptor potential cannot be evoked for the first 2 s. Responsiveness recovers within ~20 s, as shown in Fig. 6 *A*, in which pairs of orange lights with the same maximal intensity were used. The first, a long-duration light, inactivated the response. The second, a 1-s test flash, was given after variable dark intervals, and thus measured the degree of recovery of responsiveness. Fig. 6 *B* plots the recovery of responsiveness as a function of the dark interval between the adapting and the test lights. Responsiveness is presented as the ratio of the peak amplitudes of the responses to the adapting and test lights. The figure shows examples of recovery from two different flies that represent two extreme cases: a case of relatively fast recovery (triangles) and a case of relatively slow recovery (diamonds). The other five flies examined showed recovery kinetics that fell between these two extremes. In all *nss* flies, recovery was complete within 20 s.

A question arises as to whether a continuous illumination, after the decay of the response, has any effect on the following dark recovery. The bottom trace of Fig. 6 *A* shows that a sufficient dark interval between the inactivating and the test light is necessary for recovery even when the inactivating light gives no response. A long inactivating light stimulus and a subsequent short, dark interval still resulted in a

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small response to the test light, which indicates that the inactivation process is maintained during illumination in the absence of a voltage response.

Effect of Pigment Conversion on the Dark Recovery

The thermal stability of fly metarhodopsin and the large difference between the absorption spectra of the two stable states of the fly photopigment, rhodopsin (R) and metarhodopsin (M), make it possible to shift up to ~80% of the pigment mole-

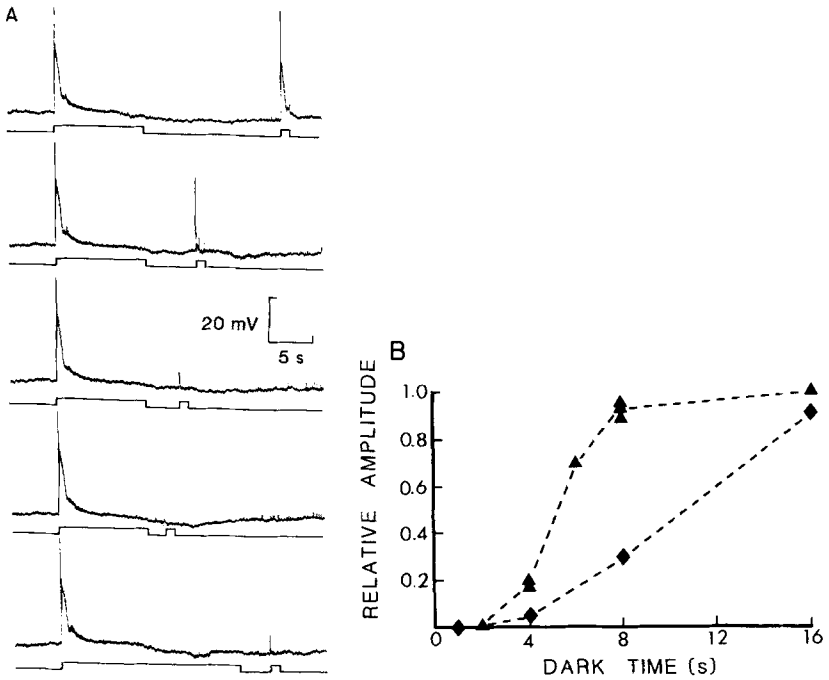

FIGURE 6. (*A*) Recovery of responsiveness of the *nss* mutant in the dark. Pairs of orange lights (OG-590) with equal maximal intensity were given to the eye. Samples of responses to these pairs, at different dark intervals, are shown. In the bottom trace, the inactivating light had twice the duration as the upper traces. (*B*) A graph showing the recovery of responsiveness in two different *nss* flies. The peak amplitude of the response to the test light was divided by the peak amplitude of the response to the longer adapting light and this ratio (relative amplitude) was plotted as a function of the dark interval between the adapting and test light. The solid triangles were measured in the same fly as in panel *A*.

cules to the M state (Hamdorf, 1979; Hillman et al., 1983; Stavenga and Schwemer, 1984). Pigment conversion from R to M in the *nss* has a very pronounced effect on the dark recovery, as shown in Fig. 7 *A*. An intense orange light pulse completely suppressed the response (upper trace, on the left). However, since the orange light does not convert pigment from R to M, a 10-s dark interval followed by a strong orange test light revealed that the responsiveness largely recovered during the dark interval (Fig. 7 *A*, upper trace). On the other hand, an intense 10-s blue light, which

changed the visual pigment composition, putting maximal pigment in the M state, largely reduced the ability of the response to recover. This is revealed by three additional intense blue test pulses that gave no responses (Fig. 7 *A*, lower trace). An additional intense orange light pulse that photoregenerated the pigment back from M to R also gave no response, but the following orange test pulses, applied after a 10-s dark interval, indicated that the responsiveness largely recovered (Fig. 7 *A*, lower trace).

The effects of pigment conversion on the dark recovery of the *nss* response are

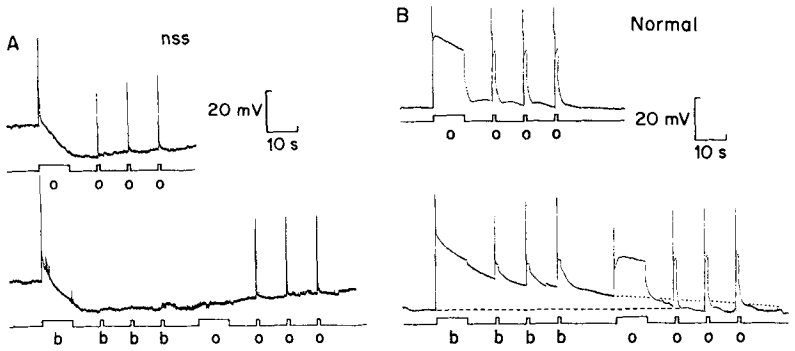

FIGURE 7. Pigment conversion from R to M induced persistent inactivation of the *nss* response in the dark, which could be abolished by M-to-R conversion. (*A*) Upper trace: receptor potentials of the *nss* mutant in response to prolonged (10 s) maximum-intensity orange light ("o"), followed by three short (1 s) orange test pulses ("o") of the same maximal intensity, interspaced by three dark periods as indicated. The responsiveness largely (by 74%) recovered after the first 10s dark period. Lower Trace: responses of the same cell to maximal intensity blue ("b") light (BG-28) followed by three additional blue test pulses ("b") of the same maximal intensity. No response was elicited by the blue test pulses. The following 10-s maximum-intensity orange light, which converted the pigment from M to R, also elicited no response. This M-to-R conversion, however, re-established responsiveness, as indicated by the appearance of responses to the three maximum-intensity orange test pulses. (*B*) Pigment conversion from R to M induced a short-lived PDA in normal *Lucilia*. The same paradigm of stimulation as in panel *A* was used to stimulate the photoreceptors of normal *Lucilia*. The upper trace shows typical responses to intense orange lights. The bottom trace shows that the initial long, intense blue light, which converted R to M, induced a PDA on which the following responses to the blue test stimuli were superimposed. The subsequent orange light, which converted M to R, suppressed the residual PDA to baseline (lower dotted line). The upper dotted line represents the level of the unaffected PDA, which was recorded in a similar paradigm of stimulation using blue lights without the orange lights (not shown).

readily explained when the same paradigm of illumination is applied to stimulate the normal *Lucilia* (Fig. 7 *B*). Fig. 7 *B* shows that orange light, which did not induce a net change in the distribution of pigment molecules between the R and M states, elicited a typical receptor potential that declined to baseline after the light was turned off. Blue light, which converted pigment from R to M, on the other hand, induced a receptor potential that did not return to baseline at the cessation of the light, but a PDA was recorded in the photoreceptor in the dark (for reviews, see

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Hamdorf, 1979; Hillman et al., 1983; Minke, 1986). This PDA is equivalent to light excitation in almost every respect (Minke, 1986). Unlike the PDA of *Drosophila,* which lasts for several hours, the PDA of *Lucilia* is relatively short and declines to about half-maximal amplitude within ~1 min. The low-amplitude PDA could be depressed to baseline when orange light was given (Fig. 7 *B*, lower trace). In the case of the *nss* (Fig. 7 *A*), a plausible explanation for the slow recovery in responsiveness after blue light is as follows. The initial blue light pulse that converted the pigment from R to M induced excitation that affected the *nss* response in the dark as if the light were still on. This pigment-induced excitation, which continued in the dark, inactivated the responsiveness and prevented its recovery. According to this explanation, pigment excitation equivalent to a low-amplitude PDA in normal *Lucilia* was sufficient to prevent the dark recovery of the *nss* response to light. The following orange light, which shifted the photopigment from M to R and depressed the dark excitation, enabled the dark recovery to occur.

The Relationship between Photopigment Conversion and Response Inactivation

Fig. 8 shows an example of the experiments in which the amount of photopigment conversion from R to M, needed to inactivate the *nss* response, was determined. Various intensities of 40-s blue lights were given to the orange-adapted eye in which all the pigment was initially in the R state. A sample of the ERG responses to the blue lights is presented in Fig. 8 *A*. Inactivation of the *nss* response is determined by the decline of the steady state phase to baseline during prolonged light. This decline to baseline can be detected very clearly in the ERG by comparing the ERG records in the dark and during the steady state response to a prolonged light pulse. In the experiment of Fig. 8 *A*, at a light intensity of $\log I_{max}/I = 2.3$, a full inactivation was observed. The relative number of M molecules created by the 40-s blue light of that particular intensity was determined by measuring the peak amplitude of the M potential in response to a constant bright orange test flash, which was applied after various intensities of the 40-s adapting blue light pulses. The M potential is a linear manifestation of the relative number of M molecules in the M state (see Pak and Lidington, 1974; Stephenson and Pak, 1980; Minke and Kirschfeld, 1980). Fig. 8 *B* shows a sample of M potentials ("Mp," arrow) elicited by the orange flash after the cessation of the adapting blue lights of various intensities as indicated. The responses of Fig. 8, *A* and *B*, were measured sequentially from the same fly. The amount of blue light needed to convert $1 - 1/e$ of the 80% convertible pigment from R to M was determined by the graph of Fig. 8 *C*. This graph plots $1 - Mp/Mp_{max}$ in log scale as a function of the amount of blue adapting light, where Mp_{max} is the peak amplitude of the M potential obtained after saturating adaptation with blue light. The ERG records showed that 40 s of maximal intensity blue light, attenuated by 2.3 log units, is required for the full inactivation of the *nss* response. From the graph of Fig. 8 *C*, we calculated that ~26% (of the total pigment molecules) were converted from R to M by that particular amount of blue light. Since at $\log I_{max}/I = 2.3$ the response declined to baseline within ~20 s of blue stimulation, conversion of ~13% of the total pigment molecules is required to inactivate the *nss* response. Similar measurements performed in the *trp* mutant of *Drosophila* indicated that ~10% of

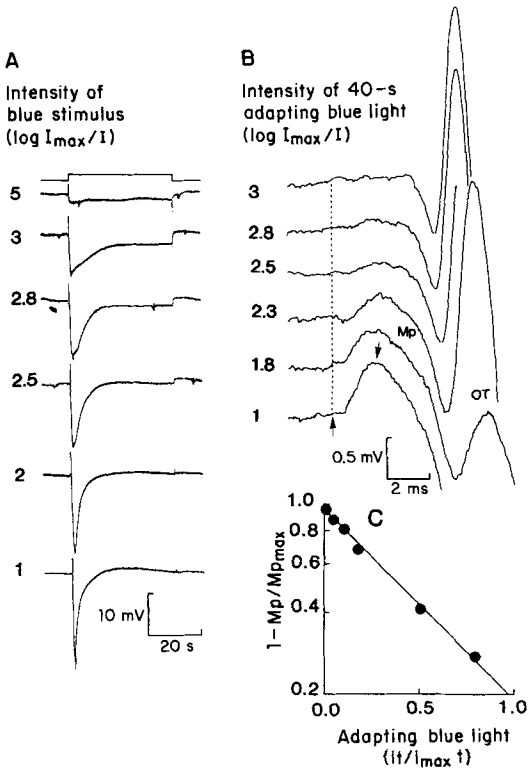

FIGURE 8. The relationship between photopigment conversion and response inactivation in the *nss* mutant. (*A*) ERGs recorded from the orange-adapted eye of the *nss* mutant in response to increasing intensities of blue (BG-28) lights with maximal intensity attenuated (in log scale) as indicated. Saturating orange adaptation, followed by a 3-min dark period, was applied before each blue stimulus. At a relative intensity of 2.5, but not at 2.0, the response did not return to baseline during stimulation and a significant steady voltage response can still be observed. (*B*) Metarhodopsin potentials (Mp) were recorded from the same eye in response to a constant, maximal intensity, orange test flash (OG-570) of 1.5 ms duration. The test flashes were applied 10 s after the cessation of the various 40-s blue lights as indicated, which elicited the responses presented in *A*. The flash was applied at the time indicated by the upward-pointing arrow and the vertical dotted line. The peak amplitude of Mp is indicated by the downward-pointing arrow. The prominent second positive phase is the on transient (OT), which arises from the lamina neurons. The graph at the bottom right (*C*) plots $1 - Mp/Mp_{max}$ as a function of the relative amounts of the adapting blue light of *A* (in linear scale), where Mp_{max} is the maximal amplitude of Mp shown in *B* (bottom, downward-pointing arrow). Increasing the amount of adapting blue light (above log $I_{max}/I = 1.0$) by a factor of 10 gave an Mp with the same amplitude as the bottom response in *B* (not shown). The relative amounts of the adapting blue lights at the various points in the graph are: 0.03, 0.05, 0.10, 0.17, 0.51, and 0.80. The response to a relative amount of adapting light of 0.80 is not presented in *B*. From the graph (*C*) it was found that the amount of blue light that inactivated the response (log $I_{max}/I = 2.3$ with a duration of 40 s and a relative amount of 0.17) converted ~26% of the total pigment molecules. Experiments were performed on four additional flies with similar (within a factor of 2) results.

the photopigment molecules have to be converted in order to inactivate the response (Minke, 1982; Minke, B., unpublished observations). It should be pointed out that, in the *nss*, as in the *trp* mutant, the phenotype of the mutant is already observed at lower light intensities than log $I_{max}/I = 2.3$.

The Effect of Background Light on the Response Latency and Rise Time

In invertebrates, the shortening of the response latency to a test pulse during a weak and medium background light intensity is a typical effect of light adaptation (Millecchia and Mauro, 1969; Brown and Lisman, 1975; Howard, 1983; Stieve et al.,

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1986). The effect of background light on the response of the normal *Lucilia* and on the response of the *nss* mutant is presented in Fig. 9. The upper traces of Fig. 9 show dark-adapted receptor potentials (elicited after a 2-min dark period) recorded intracellularly from a single photoreceptor of the *nss* mutant (left) and normal *Lucilia* (right) in response to increasing intensities of orange (OG-570) flashes as

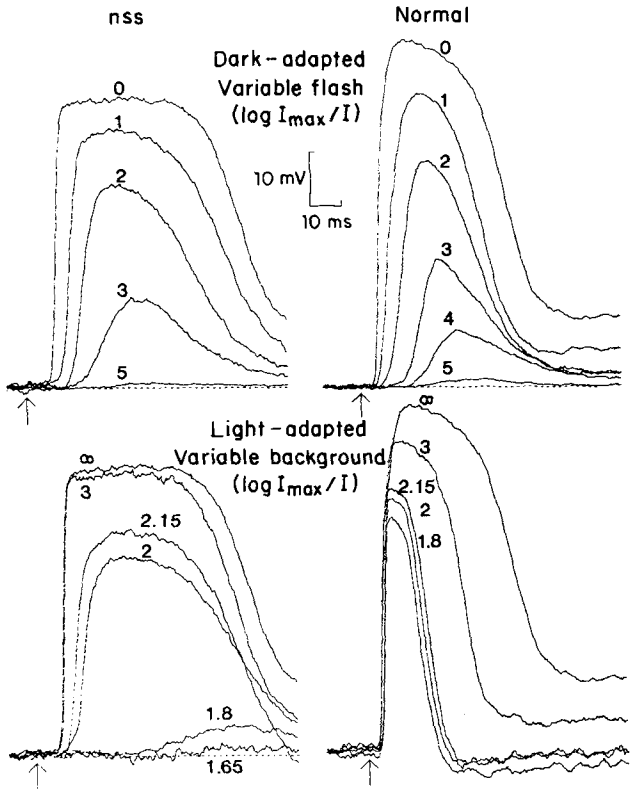

FIGURE 9. The effect of background lights on the response latency and rise time. Intracellular recordings from single photoreceptors of normal *Lucilia* (right) and the *nss* mutant (left) to increasing intensities of orange flashes (OG-570) of 1.5 ms duration with relative intensities as indicated. The upper traces were recorded from dark-adapted cells (2-min dark adaptation) and the bottom traces are from light-adapted cells 20 s after the onset of orange (OG-590) background lights with relative intensities as indicated. Three responses were averaged. The noisy recordings of the bottom traces is due to the background lights, which elicited photoreceptor noise. The arrows indicate the time when the flash was applied.

indicated. In both the mutant and the normal fly, the latency decreased with increasing intensity of the stimulus. The responses of the mutant, however, had a longer duration and latency as compared with the normal fly. The latencies of the *nss* response to light intensity of log I_{max}/I = 5.0 (or 3) and of log I_{max}/I = 0 were 27 and 6 ms, respectively. In the normal fly, the latencies to stimuli of the same intensities were 15 and 3 ms, respectively (i.e., about half the duration in the mutant).

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The bottom traces of Fig. 9 show the responses of the same cells of the mutant (left) and the normal fly (right) to a maximal-intensity orange flash that was applied 20 s after the onset of orange background lights, with the relative intensity as indicated. In the normal fly, background lights slightly shortened the latency (by ~1 ms) and mainly reduced the duration of the response in a graded manner, with no detectable difference between the effects of background lights of relative intensity 2 and 1.8 on the latency. In the mutant, on the other hand, the effect of dim light (log $I_{max}/I = 3.0$) is very small (i.e., <0.5 ms increase in latency). However, when the background light had reached the narrow range of critical intensity (>log $I_{max}/I = 2.0$), which suppressed the response to baseline, the amplitude of the response to the test flash was largely reduced, together with a very large increase in the response latency (46 and ~75 ms at background lights of log $I_{max}/I = 1.8$ and 1.65, respectively). The responses of the dark-adapted cell (left, log $I_{max}/I = 5$) had a considerably shorter latency (of 27 ms) compared with that of the light-adapted cell (left, log $I_{max}/I = 1.65$) with similar response amplitudes that had a latency of ~75 ms. Fig. 9 thus demonstrates that a dramatic increase in the response latency occurs when the receptor potential is largely reduced in amplitude. As will be discussed below, this phenomenon represents an important characteristic of the receptor potential of the *nss* mutant.

DISCUSSION

Similarities in the Effects of the nss and the trp Mutations

The effect of the *nss* mutation on the photoreceptor potential of *Lucilia* is very similar to the effect of the *trp* mutation on the receptor potential of *Drosophila* (see Howard, 1982). For both the *nss* and the *trp*, the following characteristics can be listed. (*a*) The response to a flash or to a weak steady light is close to normal, and the response decays to baseline after a relatively small increase in the steady light intensity in a critical range of light intensities. (*b*) The decline of the response is accompanied by a decrease in the rate of occurrence of the quantum bumps until individual bumps at a rate of <5/s are evident in the *nss* response. (*c*) Background light increases the response latency and rise time, whereas in the normal fly the effects are in the opposite direction. (*d*) The responsiveness recovers within 1 min in the dark after a strong adapting light is turned off. (*e*) Pigment conversion from R to M prevents the recovery of responsiveness in the dark. (*f*) Pigment conversion by a long-duration blue light that converts ~10% of the total pigment from R to M inactivates the response to light. (*g*) The decay of the response does not seem to arise from a large increase in intracellular Ca^{2+}, as suggested by measurements of pigment migration in the *nss* mutant (Howard, 1984) and by the inability of Ca^{2+} buffers to prevent the decline of the *trp* response during light (Minke, 1982).

The differences between the effects of the two mutations on the receptor potential are quantitative rather than qualitative. For example, the receptor potential of the *nss* mutant in response to prolonged intense light usually decayed well below baseline, a phenomenon that only seldomly is observed in the *trp* mutant (Minke, B., unpublished observations). This difference can be explained, for example, by a stronger activity of the electrogenic Na/K pump in *Lucilia* than in *Drosophila* in the

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penetrated cells that may be partially damaged in *Drosophila*. The dark recovery of the *nss* responsivity is about four times faster than that of the *trp*. The small differences between the effects of the *nss* and the *trp* mutations presumably involve properties of the photoreceptors of *Lucilia* and *Drosophila* that are unrelated to the mutations.

The decline of the *nss* and *trp* response to baseline during prolonged intense light cannot arise from an increase in conductance to ions like K or Cl with negative equilibrium potentials. This conclusion is based on bridge measurements in the *trp* (Minke, 1982) and *nss* (Howard, 1984) mutants, which showed that in both mutants the decline of the response is accompanied by a parallel decrease in conductance. A strong activity of the electrogenic Na/K pump during light is also not a plausible mechanism for the decline. This is because such a mechanism should not reduce the amplitude of the light-induced noise but rather increase it, contrary to the experimental observations. The stable intracellular recordings and the ability to record large light-induced voltage noise in the *nss* mutant makes it possible to examine more closely the main effect of the mutation, namely the nature of the reduction in excitation efficiency and its relation to light adaptation.

The nss Mutant Shows an Unusually Small Degree of Light Adaptation

One of the striking features of the *nss* response to light is the very small degree of light adaptation in responses produced by bright flashes or background light of medium intensity; the same lights cause considerable light adaptation in the normal fly. This small degree of light adaptation in the mutant is manifested by (*a*) a very slow response to an intense flash superimposed on background light (Fig. 9); (*b*) the appearance of bumps with similar shapes and amplitudes during the decaying response to light of medium intensity and to light of 1,000 times weaker intensity (Fig. 3); and (*c*) the small reduction in the calculated effective bump duration during light of medium intensity (Table II).

In invertebrate photoreceptors, both the rise time and the latency of the receptor potential, as well as the size and duration of the quantum bumps, are known to decrease upon an increase in the intracellular free Ca^{2+} level ($[Ca^{2+}]_i$) (Lisman and Brown, 1972; Brown and Lisman, 1975; Bader et al., 1976; Fein and Charlton, 1978; Stieve and Bruns, 1983; Stieve et al., 1986). It has been demonstrated that light causes the reduction in the amplitude and time scale of the response by increasing $[Ca^{2+}]_i$ (for reviews see Brown, 1986; Stieve, 1986). Since in the *nss* mutant light does not cause the typical changes in the amplitude and time scale of the receptor potential or quantum bumps, it might be suggested that light causes very little or no increase in $[Ca^{2+}]_i$ in the mutant. Strong support for this suggestion was provided by the experiments of Howard (1984) in the *nss* mutant. He demonstrated that, in the red-eyed *nss*, pigment migration, which is triggered by an increase in $[Ca^{2+}]_i$ during intense light (Kirschfeld and Vogt, 1980), did not occur in the mutant. A transient pigment migration during continuous light was observed only when extracellular Ca^{2+} was markedly increased, which presumably allowed some Ca^{2+} to enter the photoreceptors during the short response to light (Howard, 1984). Thus, the machinery for pigment migration is functional in the mutant but presumably does not operate, owing to the lack of increase in $[Ca^{2+}]_i$.

It has been suggested by Stieve (1985, 1986) that the shortening of the latency of the macroscopic receptor current with increasing stimulus intensity in the *Limulus* ventral photoreceptor can be explained by the bump latency distribution: higher light intensities favor the occurrence of (the more improbable) short bump latencies. The bumps' latency distribution can qualitatively explain our observation that, during background light, which reduces the rate of occurrence of the bump, the latency of the macroscopic response was prolonged. A question arises as to whether it is possible to explain the macroscopic receptor potential of the mutant by assuming that the mutant response to intense light becomes equivalent to that seen during weak light. Quantitatively, this assumption does not hold since a dark-adapted response to a weak light always has a significantly (a factor of >2) shorter latency than to the latency of the light-adapted response with a similar amplitude (Fig. 9). Thus, a dark-adapted response to a weak flash and a light-adapted response to strong light may contain a similar number of bumps of similar shapes and amplitudes; nevertheless, the manner in which these bumps are triggered and summate may be very different in the two cases. In both cases, however, light adaptation is minimal, which suggests some dependence of light adaptation on the amount of charge transferred during the response to light.

The nss Mutation Affects the Triggering Mechanism of the Bump

The calculations of the power spectra and the effective bump duration from the light-induced noise of the *nss* mutant fit nicely with our qualitative observations and strongly support our suggestion that the *nss* mutation causes a reduction in the rate of occurrence of the bumps, i.e., in the quantum efficiency to evoke bumps. The similarity in amplitude and the lack of an apparent change in the shape of the bumps before and after the decline of the response to lights of medium intensities indicates that the *nss* mutation, like the *trp* mutation, affects the mechanism of bump triggering (which includes latency; see below) and not that of the bump parameters, i.e., the bump amplitude and shape.

Several independent studies have indicated a separation between the triggering process (which includes the latency) and bump generation. Pak et al. (1976) have shown that the phototransduction mutant of *Drosophila norpA*^{H52} is defective in an intermediate process of phototransduction that affected neither the photopigment nor the bump process (Pak et al., 1976). Wong et al. (1980) have suggested, on the basis of temperature dependence of bump latencies using noise analysis, that the mechanisms underlying the bump and the latency process are different. Stieve and Bruns (1983) and Howard (1983) also have shown that, in *Limulus* ventral photoreceptors and in the locust eye, respectively, latency and bump size are not correlated. Keiper et al. (1984) have shown that the light-induced bump is characterized by four independent parameters: the latency, the slope of the rising phase, the rise time, and the time constant of the exponential decay (for a summary, see Stieve, 1986). This study, together with other theoretical studies of the bump properties (Lisman and Goldring, 1985; Schnakenberg and Keiper, 1986), also predicted that little or no gain is involved in the stages that determine the latency of the light response (in contrast to the situation of the receptor potential of vertebrate photoreceptors). These studies suggested the existence of separate independent stages for triggering

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(and latency) and for bump shape and amplitude. The fact that the *nss* mutation primarily affects the rate of occurrence of the bumps but only slightly affects their shape and amplitude provides direct experimental evidence for the existence of the hypothesized triggering stage. Further support for the existence of this separate triggering stage, which also determines the latency, came from our observation that when the response of the *nss* declined to baseline, during intense background lights, the response to a strong superimposed test flash had an unusually long latency (Fig. 9).

In the *Limulus,* the circadian clock at night makes spontaneous bumps rare and makes light-induced bumps easier to elicit (Kaplan and Barlow, 1980). This effect appears to be the exact opposite of the effect of the *nss* mutation on the light response during background light. While the effect of the clock on the rate of the light-induced bumps can be explained by changes in photoreceptor structure, the reduction in the spontaneous bump rate seems to arise from modulation of early events in phototransduction (Barlow et al., 1987). Both the circadian clock and the *nss* mutation may affect the same mechanism of bump triggering.

Conclusion

The receptor potential of the *nss* mutant of *Lucilia* is close to normal in response to dim light or short flashes but quickly declines to baseline during prolonged intense illumination. The decline of the response to baseline arises from a continuous reduction in the rate of occurrence of the quantum bumps (reduction in excitation efficiency) without much change in their effective duration or amplitude when light of medium intensity is used. The light-induced reduction in excitation efficiency, together with the large increase in the response latency to intense test flashes during background light, suggest that the *nss* mutation affects the triggering mechanism of the bumps and that this mechanism is independent of the mechanism that determines the bump amplitude and shape. The close similarities in the effects of the *nss* and *trp* mutations on the receptor potential make it possible to exploit the advantages of each of them to elucidate the molecular mechanism underlying the triggering mechanism of the bump.

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